REMARKS

Reconsideration and allowance are respectfully requested.

All previously pending claims have been canceled. New claims 18 to 21 have been submitted. The new claims find support in the specification and claims as originally filed. No new matter has been added by the present amendments to the claims.

The Objections to the Claims

The Examiner objected to certain claim language in former claims 10 to 16. These claims have been canceled and so the rejection is now moot. New claims have now been submitted and are respectfully submitted to no longer suffer from the deficiencies previously alleged by the Examiner.

The Examiner also objected to the use of hyperlinks in the specification. The hyperlinks that had been in the specification have now been deleted, and it is respectfully submitted that this ground for objection has now been obviated.

The Rejection of Claim 16 Under 35 USC 112, 1st Paragraph

Claim 16 has been deleted, and so the rejection for lack of enablement is respectfully submitted to be moot. It is respectfully submitted that all of the newly added claims are fully enabled and comply with the strictures of 35 USC 112, 1st paragraph.

The Rejections Under 35 USC 103(a)

Claims 10 to 16 have been canceled, and so the rejection of these claims as obvious is moot.

To the extent the Examiner might consider extending the same grounds for rejection to the presently pending claims, applicants have the following comments.

New claims 18 to 21 all include the limitation of former claims 11 to 13, that the poly(ethylene glycol) groups are branched. Claims 11 to 13 were not rejected over the combination of the teachings of Ashkenazi, Cox, Francis, and Byun. Accordingly, it is

respectfully submitted that the present claims also would not be properly rejected over this combination of references.

Canceled claims 11 to 13 were rejected over the combined disclosures of Ashkenazi, Cox, Francis, Byun, and Veronese. To the extent that the Examiner would consider applying the same grounds of rejection to the present claims, applicants respectfully traverse, and request reconsideration.

None of the cited references discloses or suggests that an IGFBP-4 PEGylated with a branched PEG residues of about 40 kDa has the superior properties relative to 20 kDa PEGylated IGFBP-4 as reported in the examples of the present application, and in particular none of the cited references provide any expectation that a 40kDa PEGylated IGFBP-4 would be less toxic than a conjugate with a 20 kDa PEG residue, as is demonstrated by the data in the present application.

To the extent that the Examiner would assert that PEG molecules with an average molecular weight of 20 kDa, such as those disclosed by Cox, "overlap" with the limitations of the present claims requiring that they comprise branched PEG groups with an overall molecular weight of 40 kDa, applicants respectfully submit that this position is contradicted by Fee et al., (Biotechnology and Bioengineering (2005), Vol. 98, No.4, 725-731), which reports in Table 1 on page 727 that the overall and actual molecular weights of PEGylation reagents with molecular weights from 5 to 40 kDa as measured by MALDI-TOF have a well defined molecular weight and no overlap. An IDS citing Fee et al. is submitted herewith.

As shown in Fig. 3 of the application wild type IGFBP-4 and the PEGylated forms of BP-4 have almost identical activity in cell culture assays. Therefore, the claimed modifications do not have a significant effect on the binding and inhibition of IGF.
Fig. 2 shows that the half life of PEGylated molecules is significantly prolonged in vivo. Modification with 20 kDa PEG shows significantly improved serum half live and serum levels in comparison to wild type IGFBP-4. PEGylation with 40 kDa PEG results in significantly higher serum levels and longer serum half life than observed for 20 kDa-PEG-IGFBP-4.

The improved serum kinetics of the claimed 40 kDa-PEG-IGFBP-4 results in significantly more potent inhibition of tumor growth than observed for the protein modified with 20 kDa PEG, as shown in example 14. Administration of 20 kDa-PEG-IGFBP-4 did not inhibit tumor growth, but administration of 40 kDa-PEG-IGFBP-4 caused mean tumor volume to be reduced from 287 mm3 to 163 mm3. Additionally, two tumor markers were significantly reduced by treatment with 40 kDa-PEG-IGFBP-4 but not by treatment with 20 kDa-PEG-IGFBP-4.

Furthermore, as described in example 15, significant histopathological alterations of kidney tissue was induced by 20 kDa-PEG-IGFBP-4. These alterations were not observed for 40 kDa-PEG-IGFBP-4.

The limitations of claim 19 are respectfully submitted to further distinguish from the cited prior art. Cox et al. only speculated that the substituted cysteine 98 or 101 may not have free thiols for reaction with PEG reagent because mixed disulfides with glutathione can be formed during refolding. In a later publication by Van Den Berg and Cox et al. (1997, European J. of Cancer 33/7, 1108-1113) on the PEGylation of the Cys 101 mutant the authors state that Cys101 of the refolded protein is free and does not participate in disulfide bonds. Serine101 in this position of the wt protein is a major site of phosphorylation. Therefore, Cox et al. have not proven that the conditions applied are really suitable for reduction of the artificial cysteines.

The stability of the Cys110-Cys117 disulfide bond of IGFBP-4 and the conditions for selective reduction cannot a priori be transferred from experiments with artificial cysteines 98 or 101 in IGFBP-1. The positions in IGFBP-1 were chosen because Ser101 is a major phosphorylation site which is usually exposed on the surface of the protein and not involved in disulfide bonds. Mixed disulfides of cysteines in this position with glutathione are artificial, not stabilized by secondary and tertiary structure and are more sensitive to reduction than natural disulfide bonds.

In contrast, Cys 110 and 117 of IGFBP-4 form a natural disulfide bond in the wild type protein and reduction of a natural disulfide bond might interfere with the stability of other natural disulfide bonds in the protein. Therefore, it can not be concluded that the

disulfide bond in IGFBP-4 can be selectively reduced under similar conditions as potential mixed disulfides with artificial cysteines in IGFBP-1.

Finally, it is respectfully submitted that even some of the art cited by the Examiner argues in favor of the nonobviousness of the present claims. Francis posits that "PEGylation of proteins is always based on trial and error and virtually all parameters of such a PEGylation can have a surprising and very profound effect on the functionality of the product" (specification, paragraph 6).

In light of the above arguments and amendments, it is respectfully submitted that the newly submitted claims are in condition for allowance, and such action is earnestly solicited.

No further fee is required in connection the filing of this Amendment. If any additional fees are deemed necessary, authorization is given to charge the amount of any such fee to Deposit Account No. 08-2525.

Respectfully submitted,

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